Inflammation-induced sensitization of the brain in term infants

BOBBI FLEISS[1,2,3,4,5] | CALLY J TANN[6,7] | VINCENT DEGOS[1,2,3,4] | STÉPHANIE SIGAUT[1,2,3,4] | JULIETTE VAN STEENWINCKEL[1,2,3,4] | ANNE-LAURE SCHANG[1,2,3,4] | ANTON KICHEV[5] | NICOLA J ROBERTSON[6] | CARINA MALLARD[8] | HENRIK HAGBERG[5,9] | PIERRE GRESSENS[1,2,3,4,5]

1 Inserm, U1141, Paris; **2** University Paris Diderot, Sorbonne Paris Cité, UMRS 1141, Paris; **3** Department of Child Neurology, APHP, Robert Debré Hospital, Paris, **4** PremUP, Paris, France. **5** Division of Imaging Sciences, Department of Perinatal Imaging and Health, King's College London, King's Health Partners, St. Thomas' Hospital, London; **6** Institute for Women's Health, University College London, London; **7** Clinical Research Department, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK. **8** Department of Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg; **9** Department of Obstetrics and Gynaecology, Sahlgrenska University Hospital, Gothenburg, Sweden.

Correspondence to Pierre Gressens, Inserm U1141, Hôpital Robert Debré, 48 Blvd Serurier, 75019 Paris, France. E-mail: pierre.gressens@inserm.fr

PUBLICATION DATA
Accepted for publication 24th November 2014.
Published online

ABBREVIATIONS

COX	Cyclooxygenase
GRK	G protein-coupled receptor kinase
HDAC	Histone deacetylase
LPS	Lipopolysaccharide
mGluR	Metabotropic glutamatergic receptor
PGE_2	Prostaglandin E_2
TLR	Toll-like receptor
TNF	Tumour necrosis factor

Perinatal insults are a leading cause of infant mortality and amongst survivors are frequently associated with neurocognitive impairment, cerebral palsy (CP), and seizure disorders. The events leading to perinatal brain injury are multifactorial. This review describes how one sub-injurious factor affecting the brain sensitizes it to a second injurious factor, causing an exacerbated injurious cascade. We will review the clinical and experimental evidence, including observations of high rates of maternal and fetal infections in term-born infants with neonatal encephalopathy and cerebral palsy. In addition, we will discuss preclinical evidence for the sensitizing effects of inflammation on injuries, such as hypoxia–ischaemia, our current understanding of the mechanisms underpinning the sensitization process, and the possibility for neuroprotection.

PERINATAL BRAIN INJURY IN THE TERM-BORN INFANT

Intrapartum neonatal deaths are the third leading cause of global childhood mortality.[1] In addition, each year perinatal insults are estimated to be responsible for more than one million new cases of neonatal encephalopathy, and nearly half a million infants with impairments such as cerebral palsy (CP).[2] Infants exposed to a perinatal insult typically present with encephalopathy, a descriptive term for a clinical constellation of neurological dysfunctions in the term-born that includes difficulty with initiating and maintaining respiration; depression of tone and reflexes; subnormal levels of consciousness; and seizures.[3] Whilst often assumed to result solely from acute intrapartum hypoxic–ischaemia, the precise contribution of hypoxia is likely to vary according to the definition of encephalopathy used, the range of competing risks, and the care setting.[3,4]

Presumed hypoxia–ischaemia is diagnosed based on reduced Apgar scores and acidosis,[5] and the combination of progressive hypoxia, hypercarbia, and acidosis is often referred to as asphyxia.[6] Encephalopathy resulting from hypoxia–ischaemia can be clearly diagnosed only in a small number of cases in which a sentinel event, such as placental abruption, uterine rupture, cord prolapse, or shoulder dystocia, is clearly present. Clinical studies have identified a number of other important antepartum and intrapartum risk factors for neonatal encephalopathy.[7–10] In low-income settings, where access to skilled birth attendants and emergency obstetric intervention is often limited, the probability of intrapartum hypoxic events contributing to neonatal encephalopathy is increased.[2,9,11] However, in general, neonatal encephalopathy is likely to be a multifactorial condition with complex aetiology, encompassing several causal events, with strong evidence that fetal exposure to infection contributes.[12]

CONCEPT OF INFLAMMATION-INDUCED SENSITIZATION

An increasingly common model for the complex and multifactorial process of perinatal brain injury involves sensitization,[13–15] whereby factors not severe enough by themselves to induce significant brain damage make the developing brain more susceptible to a second insult (Fig. 1). Substantial numbers of preclinical studies have demonstrated the

© The Authors. Journal compilation © 2015 Mac Keith Press

sensitizing effects of gestational or neonatal systemic inflammation, gestational chronic mild maternal stress, and gestational hypoxia on perinatal excitotoxic or hypoxic–ischaemic lesions.[16–21] Genetic factors have also been shown to influence the developing brain's response to sensitizing factors.[22,23]

Robust epidemiological studies, performed in preterm and term-born neonates, support a role for inflammation as a sensitizer. These studies suggest a strong association between fetal infection and inflammation (e.g. chorioamnionitis) and perinatal brain damage and neurological disability (as discussed in more detail below).[24–26] Altogether, these data support the hypothesis that factors such as infection and inflammation can sensitize the developing brain to injurious events occurring during or after birth. We believe that this complex process significantly contributes to the neurological prognosis of neonatal encephalopathy. In this review, the experimental and clinical evidence for sensitization, what we have learned about the mechanisms underpinning neonatal encephalopathy, and the possibility for neuroprotection are discussed.

CLINICAL OBSERVATIONS ON INFLAMMATION-INDUCED SENSITIZATION TO HYPOXIC–ISCHAEMIC INJURY

In a term pregnancy, the fetal membranes usually act to provide an effective barrier to ascending bacterial infection from the lower reproductive tract. However, infection and inflammation of the placental membranes (chorioamnionitis) may occur, most frequently in the presence of intrapartum complications such as a prolonged labour or rupture of membranes. Clinical chorioamnionitis is characterized by maternal fever, uterine tenderness, foul-smelling liquor, and the presence of raised maternal inflammatory markers, but is often poorly defined because of the heterogeneous nature of these symptoms and signs.[27] Conversely, histological chorioamnionitis is clearly defined, by microscopic evaluation, as neutrophilic granulocytes in the chorionic plate or extraplacental membranes. In isolation, histological chorioamnionitis is a frequent subclinical infection.[28] Neither maternal fever nor clinical chorioamnionitis has been found to accurately predict histological findings.[29] In its severest form, membrane inflammation involves the cord (funisitis), with vasculitis of the umbilical vessels and the presence of fetal neutrophilic granulocytes indicating activation of the fetal inflammatory response. Fetal inflammatory responses have been associated with neurological impairment in both term-born and preterm infants.[30]

Observations on maternal fever

Maternal fever has been shown to increase the risk of neonatal encephalopathy, although it does not correlate with long-term outcomes.[8,9,29] In a prospective cohort study, maternal fever (≥37.5°C) was associated with an increased risk of neonatal encephalopathy (odds ratio [OR] of 10.8, 95% confidence interval [CI] 4–29.3).[31] However, there is evidence that this risk may be increased in the presence of chorioamnionitis and fetal acidosis.[32–34] Hyperthermia has been shown to increase brain injury in animal models, raising concerns that fever from non-infective causes, such as epidural anaesthesia, as well as infective causes, could have deleterious effects on the brain.[35]

Observations on cytokines/chemokines

Both infection and hypoxia–ischaemia trigger the fetal inflammatory response, increasing pro-inflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor (TNF) α.[35,36] This fetal inflammatory response syndrome, characterized by systemic activation of the fetal immune response, was originally defined in fetuses exposed to preterm labour. Fetal inflammatory response syndrome is known to contribute to preterm brain injury, including periventricular white matter injury.[14,37] Despite being very different types of injury, hypoxia and infection can individually trigger a cytokine response. Correlations observed between CP and the blood cytokine levels of newborn infants born at term suggest that a cytokine response may represent activation of a common

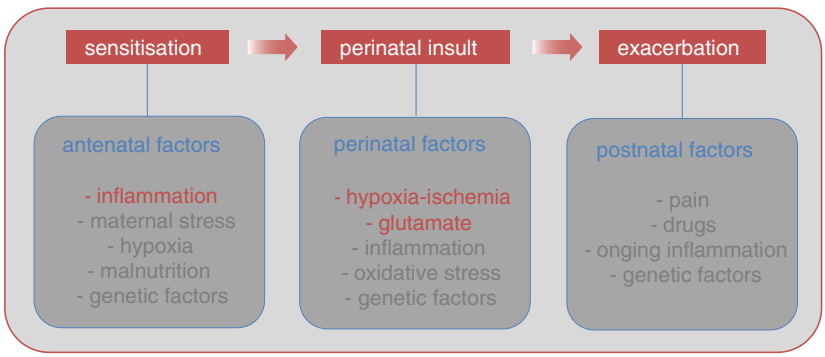

Figure 1: Schematic representation of the multiple-hit hypothesis in which different antenatal factors are predicted to make the developing brain more susceptible (sensitized) to neonatal insults. In addition, some factors occurring in the neonatal intensive care unit could further enhance brain damage (exacerbation).

inflammatory pathway.[24] Importantly, cytokine levels (IL-6, IL-8, RANTES) 6 hours after birth are significantly higher in term-born infants with clinical chorioamnionitis and encephalopathy or seizures than in infants with clinical chorioamnionitis without encephalopathy or seizures.[38] However, the exact mechanisms for this association are unclear.[38]

Observations on maternal and newborn sepsis

Few studies to date have examined the role of neonatal sepsis in neonatal encephalopathy. A population-based study from South Asia examining the 'dual hit' hypothesis also reported significantly increased odds of early neonatal death due to intrapartum asphyxia in newborns exposed to prolonged rupture of membranes (OR 1.52, 95% CI 1.15–2.02), an established predisposing factor for maternal and early newborn sepsis.[39] In addition, previous studies in high–income settings have shown high prevalence of inflammatory indicator (24%) amongst infants with neonatal encephalopathy.[40] A recent US study, examining the association between maternal and neonatal infections and adverse outcomes after neonatal encephalopathy, reported an increase in adverse neurodevelopmental outcomes, but not death, amongst encephalopathic infants with sepsis; however, chorioamnionitis was associated with a lower risk of brain injury and adverse cognitive outcomes were less severe in infants exposed to chorioamnionitis.[41] Although it seems likely that the burden of infectious comorbidity is highest in low-income settings, a high incidence of sepsis has been seen in many of the hypothermia trials preformed in high-income settings (5–14%).[42]

Observations on placental pathology

The potentially important role of placental pathology in the aetiology of neonatal encephalopathy was highlighted by Badawi et al.[8] in Western Australia, where the presence of an abnormal placenta was found to double the risk of neonatal encephalopathy. In addition, clinical chorioamnionitis was associated with a more than fivefold increased risk of neonatal encephalopathy by Blume et al.[29] in a retrospective, population-based study; however, histological findings were not reported. A recent study from the Netherlands examined the associations between placental pathology and the patterns of brain injury, as detected by magnetic resonance imaging (MRI), amongst term-born infants diagnosed with neonatal encephalopathy.[43] The authors reported a high incidence of histological chorioamnionitis amongst infants with neonatal encephalopathy, compared with healthy term-born infants, in a historic cohort from the same centre (50% vs 18%, respectively). Chorioamnionitis was common for all patterns of brain injury, with the highest incidences occurring in infants with the most extensive injuries and those without brain injuries.

A number of studies have shown a positive association between placental or membrane infection and inflammation, or elevated cytokine expression, and long-term neurological impairment in term-born infants.[44] Of particular note is a meta-analysis of studies examining the association between chorioamnionitis and CP, which suggests that clinical chorioamnionitis significantly increases the risk of CP amongst term-born infants, based on combined data from two small studies (relative risk [RR] 4.7; 95% CI 1.3–16.2).[45] Only one study examined the association between CP and histological chorioamnionitis, reporting a relative risk of 8.9 (95% CI 1.9–40); however, this positive association is also reflected by data from cases reviewed for medico-legal consultation, which revealed strong correlations between severe histological fetal chorioamnionitis and CP amongst infants with neurological impairments (OR 13.2, 95% CI 1.2–144).[46] In addition, a study by Nelson and Grether[33] showed that a combination of antenatal infection and a potentially asphyxiating condition at birth dramatically increases the risk of CP (OR 78) compared with the risk associated with either a potentially birth-asphyxiating condition (OR 2.5) or infection (OR 7.2) alone.

High incidences of funisitis have been reported amongst infants with neonatal encephalopathy (22–26%).[47–50] Funisitis was found to be a significant risk factor for moderate to severe neonatal encephalopathy (OR 9.29, 95% CI 1.36–63.06) in a retrospective case–control study carried out in Dublin.[49] These findings are supported by our previous work in Uganda,[48] suggesting that the association between funisitis and neonatal encephalopathy may be common across a variety of different resource settings. A more significant association between neonatal encephalopathy and funisitis, than between neonatal encephalopathy and chorioamnionitis, suggests that the duration of exposure to inflammation, or even the proximity of inflammation to the fetus, may be important. Placental histology in asphyxiated newborn infants meeting the criteria for therapeutic hypothermia was assessed in a small study.[50] The findings from this study suggest that therapeutic hypothermia might not be effective in asphyxiated newborn infants whose placentas show evidence of chorioamnionitis with fetal vasculitis; however, this needs to be confirmed in larger studies. In addition, a high mortality rate was seen in cooled infants in a cooling feasibility study carried out in a Ugandan hospital where high rates of chorioamnionitis and funisitis have been reported in encephalopathic neonates.[51]

Previous studies have shown an association between inflammation of the placental villous tree (villitis) and neonatal encephalopathy, but the mechanisms underlying this association are poorly understood.[15,21] Villitis is most frequently of unknown aetiology and is characterized by mixed lymphohistiocytic infiltration of the placenta by maternal CD-8-positive T-lymphocytes.[52] This can lead, in its severest form, to an obliterative fetal vasculopathy, shown to be associated with intrauterine growth restriction and hypertension in pregnancy, which are both independent risk factors for neonatal encephalopathy.[53] The role of the fetal cytokine response in the aetiology of villitis, and its apparent association with neonatal encephalopathy, is, to date, uncertain.

EXPERIMENTAL EVIDENCE OF INFLAMMATION-INDUCED SENSITIZATION OF THE BRAIN IN TERM-BORN INFANTS

The experimental demonstration of inflammation-induced sensitization of the brain in term infants is based on the following two rodent models of brain damage, which mimic lesions observed in term-born infants (i.e. a predominance of grey matter damage): (1) a hypoxic–ischaemic model produced by the combination of global hypoxia with a unilateral carotid artery ligation at postnatal day 7 in rats or day 9 in mice;[54,55] and (2) a model of excitotoxic brain damage produced by intracerebral injection of newborn mice or rats with the glutamatergic analogue ibotenate.[56,57]

In the hypoxic–ischaemic model, systemic injection of low and non-injurious doses of a toll-like receptor (TLR) 4 or TLR3 agonist (*Escherichia coli*-derived lipopolysaccharide [LPS] or polyinosinic:polycytidylic acid, respectively), 4 hours before a typically non-injurious hypoxic–ischaemic insult (lasting 20min), induced massive hemispheric brain damage.[21,58] Administering an agent with anti-inflammatory properties, or disabling the TLR inflammatory pathway, reduced the severity of the LPS sensitization.[59,60] These studies demonstrate that exposure to inflammation prior to a moderate hypoxic–ischaemic insult is sufficient, through a sensitization process, to make the newborn brain extremely susceptible to damage.

In the excitotoxic model, systemic injection of IL-1β, IL-6, TNFα, or IL-9 in newborn mice or rats, between postnatal days 1 and 5, makes the brain much more sensitive to brain insults induced by ibotenate on postnatal day 5, with up to twice the level of brain damage observed in these rodents compared with that observed in non-sensitized animals.[20,61] Similarly, delivery of LPS to pregnant rats on the last 2 days of gestation exacerbated ibotenate-induced brain damage in postnatal day 4 pups, with the highest dose of LPS doubling the lesion size.[17]

Several studies have evaluated the time window during which sensitization of the brain will persist following exposure to inflammatory factors in the perinatal period. Sensitization to hypoxic–ischaemic damage following neonatal administration of LPS appears to last at least up to 3 days in rats[62] and 14 hours in mice.[59] In additional experiments, we showed that intrauterine exposure of pregnant mice to LPS resulted in increased cerebral vulnerability of pups to hypoxia–ischaemia up to 14 days postnatally.[16] However, it has become evident that within a certain time of LPS exposure the brain becomes less vulnerable or, so-called, preconditioned. Thus, when LPS was administered 24 hours before injurious hypoxia–ischaemia (for 50min), brain injury was markedly reduced.[62] This observed preconditioning has also been confirmed by others.[63]

MECHANISMS OF INFLAMMATION-INDUCED SENSITIZATION

The mechanisms by which sensitization occurs are not fully understood. However, by using animal models, different key elements of this process have recently been unravelled. These new findings are improving the outlook for the design of novel therapeutic approaches (as discussed in the following sections).

Prostaglandins/cyclooxygenase-2

Systemically administered IL-1β induces central excess production of prostaglandin E_2 (PGE_2), and cyclooxygenase (COX) inhibitors block both this PGE_2 production and the sensitization effect of IL-1β on excitotoxic brain damage, suggesting a key role for the COX–PGE_2 pathway in the sensitization process.[64] Systemic IL-1β also induces an imbalanced central production of pro- and anti-inflammatory cytokines, with an increase in the production of IL-1β, IL-6, IL-18, and TNFα that is not counterbalanced by an increase in the production of IL-10.[64,65] COX inhibitors largely block these effects of IL-1β on brain cytokines. Prostaglandins may also be important in LPS-induced brain damage, as injury is associated with an increase in COX-2.[66] Furthermore, both IL-1β[67] and TNFα[68] have been implicated as possible mediators of LPS-induced damage. Recent data also support an important role for microglial recruitment and activation, as well as a role for early neutrophil infiltration, in this neuroinflammatory milieu.[69,70] Whether or not the COX–PGE_2 pathway mediates this microglial and neutrophil recruitment remains to be determined.

Glucocorticoids

There is a close interplay between the immune system and the hypothalamo–pituitary–adrenal axis. Injection of pregnant rodents with LPS is often used as a model for stress, with important modifications of the hypothalamo–pituitary–adrenal axis.[71] We have shown that mild chronic stress during murine gestation sensitizes the neonatal brain to excitotoxic lesions in a manner comparable to systemic injection with cytokines.[18] Preliminary data show that administration of RU486, a glucocorticoid and progesterone receptor blocker, completely prevents the sensitizing effect of systemic IL-1β (Fig. 2), suggesting an involvement of the hypothalamo–pituitary–adrenal axis (and/or progesterone receptors) in the sensitizing effect of systemic inflammation. In support of this hypothesis, glucocorticoids mediate LPS-induced hypoxic–ischaemic preconditioning,[72] and dexamethasone prevents the injurious effects of LPS-induced sensitization to hypoxia–ischaemia (Table I).[73]

mGluR/GRK2

At the neuronal level, increased group I metabotropic glutamatergic receptor (mGluR) activity appears to be a key molecular event underlying IL-1β-induced sensitization to excitotoxicity.[74] The activities of mGluRs are regulated by G protein-coupled receptor kinase 2 (GRK2) through phosphorylation and subsequent desensitization.[75] Uncoupling and internalization of G protein-coupled receptors are crucial regulatory processes that ensure attenuation of signalling, preventing overstimulation and damage to cells.

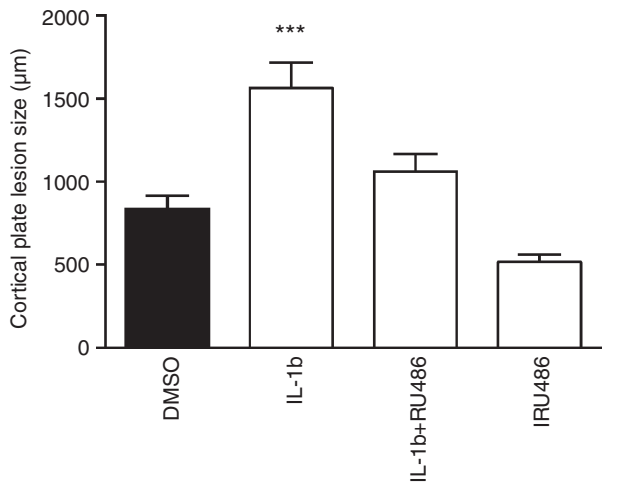

Figure 2: RU486 blocks the sensitization effect of systemic IL-1β on neonatal excitotoxic lesions in mice. Quantitative analysis of cortical lesions produced by intracerebral injection of ibotenate on postnatal day 5, and assessed on postnatal day 10. Bars represent mean and SEM values for the length of the lesion along the sagittal fronto–occipital axis. ***Statistically significant difference ($p<0.01$, as determined by analysis of variance with Dunnetts multiple comparison test) compared with control (dimethylsulphoxide)-treated mice, as represented by black bar (F Plaisant and P Gressens, unpublished material).

Inflammation downregulates GRK2,[74,76] and deletion of the gene encoding GRK2 mimics the sensitizing effect of inflammation on excitotoxic neuronal cell death,[74,77] suggesting that GRK2 is a potential molecular link between inflammation and mGluR-mediated sensitization (Fig. 3).

p53

Several mechanisms may be involved in LPS-induced sensitization. LPS binds to TLR4, which triggers Myd88-dependent induction of NFκB.[21,59,78] In addition to inducing multiple cytokines (including some of those discussed above), NFκB evokes p53 production. p53 is a tumour suppressor that triggers apoptosis (by transactivating pro-apoptotic and repressing anti-apoptotic genes), causes cell cycle arrest, and inhibits or stimulates autophagy.[79] Furthermore, p53 has cytosolic actions and can promote Bax-dependent mitochondrial permeabilization, leading to cell death,[80] a process which is known to occur in the immature brain.[81] It is possible that LPS induces NFκB expression and thus provokes p53 activation in neurons and oligodendroglial precursors, which may, in turn, cause cell death or cell cycle arrest, hampering myelination and brain recovery.[82] In support of the therapeutic potential of NFκB inhibition, attenuated NFκB signalling (by intranasal tat-NBD peptides) decreased microglial activation and brain damage following LPS-sensitized hypoxic–ischaemic damage.[83] In addition, NFκB inhibition following hypoxia–ischaemia prevents cytoplasmic and mitochondrial p53 accumulation and mitochondrial release of cytochrome C in neurons.[81] However, it remains unknown whether or not inflammatory pre-exposure enhances the involvement of p53. At time points when LPS is known to sensitize the immature brain (2h and 6h following LPS exposure), we found changes in the expression of multiple genes related to p53, NFκB, and apoptotic signalling in general (Table II).[62,84] It remains to be demonstrated, however, whether or not these pathways are involved in augmenting CNS vulnerability.

Death receptors

A primary event of neuroinflammation is the activation of microglia, which includes the release of various tumour necrosis ligands (e.g. TNFα, TNFβ, FasL, TRAIL, and TWEAK).[85–90] The interaction of these ligands with their receptors, including TNF receptor 1, FasR, death receptor 4, death receptor 5, and TWEAKR, expressed by oligodendrocyte progenitor cells and neurons, can trigger apoptotic cell death. Death receptor activation triggers an extrinsic pathway of caspase-dependent cell death, or produces truncated Bid, leading to enhancement of Bax-dependent mitochondrial pore formation and cell death. TNF receptor 1 is critical for LPS-mediated sensitization to oxygen glucose deprivation in vitro.[91] Moreover, deletion of the TNF gene cluster abolishes LPS-mediated sensitization of the neonatal brain to a hypoxic–ischaemic insult.[68]

TNFα treatment is toxic to oligodendrocyte precursors[92] and potentiates IFNγ-induced toxicity to these cells in vitro.[93] TNFα also stimulates proliferation, and potentiates the activation of astrocytes[94] and microglia.[95] The Fas receptor is upregulated in oligodendrocyte precursors exposed to an inflammatory stimulus,[93] and this may explain, in part, the inflammation-induced sensitivity of these cells to Fas-induced apoptosis. An alternative hypothesis for death receptor-mediated sensitization, suggested by Genc et al.,[96] postulates that TLR4 activation of MyD88/NFκB in microglia leads to the production of the death receptor ligands TRAIL and TWEAK. TRAIL and TWEAK have been shown to be important mediators of cell death in adult models of injury.[88,97] Recently, we have published evidence implicating these proteins as mediators of neonatal hypoxia–ischaemia,[98] and another study has implicated them as regulators of cell cycle arrest and neurite outgrowth. The latter functions can be attributed to the anti-apoptotic effects exerted by TNF receptor 2[99] and TWEAKR.[100] Several genes encoding factors involved in the death receptor pathway are regulated during LPS-induced sensitization (Table II).[84]

TLRs and cytokines

Data suggest that TLR pathway activation, leading to cytokine production, is involved in inflammation-induced sensitization to hypoxia–ischaemia. LPS-induced sensitization is dependent on TLR4,[101] in an MyD88-dependent manner.[59] More recently, other innate immunity receptors have also been implicated, including TLR3 and the downstream adaptor molecule TRIF.[58] Systemic administration of IL-9

Table I: Effects of neonatal IL-1β exposure on cortical plate-associated genes in mice

Gene name	Fold change at P5	Fold change at P10	Protein name and function
Jakmip1	1.37	1.15	Janus kinase and microtubule-interacting protein 1 associates with microtubules and may play a role in the microtubule-dependent transport of the GABA-B receptor
Jakmip2	1.03	1.27	Janus kinase and microtubule-interacting protein 2 negatively regulates transit of secretory cargo by acting as a structural scaffold of the Golgi
Cspp1	1.36	1.29	Centrosome- and spindle pole-associated protein 1 interacts with centrosomes and microtubules and plays a role in cell-cycle progression and spindle organization
Snap23	1.32	1.30	Synaptosome-associated protein 23 is an essential component of the high-affinity receptor for the general membrane fusion machinery, and is an important regulator of transport vesicle docking and fusion
Chd7	1.52	1.32	Chromodomain helicase DNA binding protein 7 is important for chromatin remodelling and is strongly associated with CHARGE syndrome
Eml5	1.16	1.78	Echinoderm microtubule-associated protein-like 5 is a microtubule binding protein
Cep350	1.16	1.34	Centrosomal protein 350 is required for anchoring microtubules to the centrosomes and for the integrity of the microtubule network that recruits PPARα, thereby modulating PPARα activity
Cep110	1.14	1.30	Centrosomal protein 110 is necessary for centrosome duplication at different stages of procentriole formation, and has a role in regulating cytokinesis and genome stability
Spin3	1.32	−1.36	Spindlin family member 3 associates with the meiotic spindle and is modified by phosphorylation in a cell-cycle-dependent fashion
Tmem14	−1.13	−1.30	Transmembrane protein 14A, mutations of which are associated with juvenile myoclonic epilepsy
Tmem88b	1.08	−1.38	Transmembrane protein 88B is a Dvl-binding protein with a role in Wnt signalling
Tspan15	−1.01	−1.36	Tetraspanin 15 mediates signal transduction events and plays a role cell development, activation, growth, and motility
Gabrd	−1.17	−1.33	GABA A receptor, δ subunit, is associated with susceptibility to generalized epilepsy with febrile seizures
Ntsr1	−1.27	1.12	Neurotensin receptor 1 belongs to the large superfamily of G protein-coupled receptors
Nxph1	−1.05	1.33	Neurexophilin 1 is expressed mainly by cells that resemble inhibitory interneurons
Pcdh15	1.08	1.33	Protocadherin 15 is an integral membrane protein that mediates calcium-dependent cell–cell adhesion
miR-411	1.14	1.41	MicroRNA 411 is associated with neuron-derived microvesicles

Frontoparietal cortical samples (n=6) from mice treated from P1 to P5 with IL-1β or vehicle control (phosphate-buffered saline) were collected at P5 and P10 and analysed using Affymetrix microarrays (Miltenyi Biotec, Paris, France). Genes were analysed and t-tests (corrected using the Benjamini–Hochberg method) were performed. Those genes significantly up- and down-regulated (>1.2-fold, p<0.05) are marked in dark grey or light grey, respectively. P, postnatal day; GABA, γ-aminobutyric acid.

in neonates has also been shown to induce sensitization to excitotoxic brain damage.[61] IL-9 is a Th2 cytokine and was found to be elevated in the plasma of a subset of neonates who later developed CP and did not have elevated levels of pro-inflammatory cytokines.[24] The sensitizing effects of IL-9 involve TGFβ-dependent mast cell activation and the release of histamine, which acts on neuronal H1 and H2 receptors.[61,102]

TARGETING INFLAMMATION TO PROTECT FROM HYPOXIC–ISCHAEMIC INJURY IN THE IMMATURE BRAIN

Efforts to design therapies aimed at reducing the sensitizing effects of inflammation have been undertaken. We will discuss some recent clinical and preclinical studies, but also point readers towards more expansive reviews on the specific topic of neuroprotection for the developing brain.[103–105]

Clinical and translational observations

There has been considerable interest in magnesium sulphate because, as well as reducing excitotoxic damage by binding to the magnesium site on the *N*-methyl-D-aspartate (NMDA) receptor, there is evidence that it can reduce secondary inflammation and associated brain injury.[106,107] There is now evidence from a meta-analysis of randomized controlled trials of infants born preterm that antenatal administration of magnesium is associated with a reduced risk of CP in early childhood.[108]

In term-born infants with neonatal encephalopathy, a key mechanism of tissue protection by hypothermia is the inhibition of the pro-inflammatory cascade induced by hypoxia–ischaemia.[109] However, the pro-inflammatory response may not be entirely harmful; some reactions are helpful for tissue repair, and hypothermia is likely to inhibit both beneficial and detrimental inflammatory responses.[110–112]

A recent study investigated the effects of therapeutic hypothermia on the peripheral immune response, within 72h of birth, in 65 infants with neonatal encephalopathy.[113] Hypothermic infants had lower white blood cell counts and lower absolute neutrophil and lymphocyte counts than normothermic infants. In the hypothermic group, those infants who did not show recovery in their white blood cell counts after rewarming had poor out-

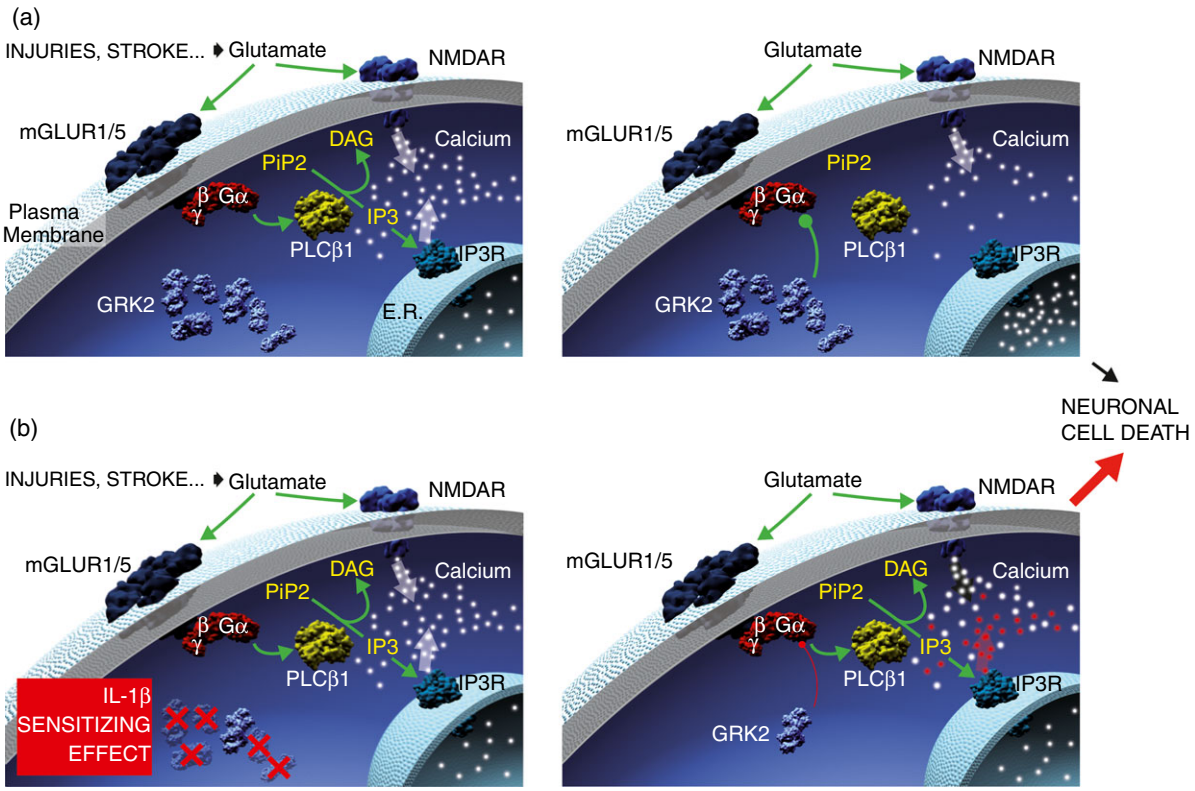

Figure 3: Schematic representation of the potential mechanisms by which group I mGluRs and GRK2 mediate IL-1β-induced sensitization. Adapted from Degos et al.[74] The figure depicts the molecular mechanisms leading to excitotoxic neuronal cell death in (a) the absence or (b) the presence of IL-1β sensitization. (a) In the absence of IL-1β, GRK2 leads to a rapid desensitization of mGluR1/5, limiting PLCβ1-mediated calcium release from endogenous stores. (b) Exposure to IL-1β leads to a reduction in GRK2, preventing the complete desensitization of mGluR1/5 and allowing a more prolonged calcium release from endogenous stores. This enhanced calcium mobilization exacerbates neuronal cell death. E.R, endoplasmic reticulum; NMDAR, N-methyl-D-aspartate receptor; mGLUR, metabotropic glutamate receptor; IP3, inositol trisphosphate; IP3R, inositol trisphosphate receptor; PiP2, phosphatidyl inositol bisphosphate; PLC, phospholipase C; DAG, diacylglycerol; G, G protein; GRK2, G protein-coupled receptor kinase 2.

comes, whereas those who had a better recovery of white blood cell counts had better long-term outcomes.[113] This may indicate immune paralysis in the adverse outcome group. It is possible, therefore, that hypothermia can have a negative influence on infants if it causes further immunosuppression. Other neuroprotective agents may be safer and more effective in inflammation-sensitized neonatal encephalopathy. For example, melatonin (*N*-acetyl-5-methoxytryptamine) enhances the immune response under immune-compromised conditions, and is a potent neuroprotectant and anti-inflammatory in animal models of brain injury, including perinatal hypoxia–ischaemia,[114] stroke,[115] and brain infection.[116]

Preclinical studies

Inflammation has been successfully reduced, in various adult and in vitro systems, by using histone deacetylase (HDAC) inhibitors, which reduce the activation of molecules such as p53 and NFκB.[117–120] Our research group demonstrated, for the first time, that HDAC inhibition can also protect the brain in a model of inflammation-sensitized hypoxia–ischaemia.[60] This proof-of-concept study demonstrated reduced brain injury and improved behavioural outcomes with a relatively broad spectrum HDAC inhibitor. It is hoped that the development of HDAC isoform-specific antagonists will increase the translational potential of this approach in the future.[121,122] Other studies have shown that inhibition of inflammation by intracerebroventricular injection of a stable, mutant form of plasminogen activator protein-1, given up to 4 hours after the onset of LPS-sensitized hypoxia–ischaemia, reduced hypoxia–ischaemia-induced blood–brain barrier damage, microglial activation, and brain tissue loss.[123] Furthermore, we recently demonstrated that blocking sensitizing inflammation with a novel class of innate defence regulator peptide (IDR-1018) provided marked neuroprotection and modulated inflammation. This effect was observed even when the peptide was given 3 hours after the induction of LPS-sensitized hypoxia–ischaemia. Bioinformatic network analysis revealed that treatment with IDR-1018 before the onset of hypoxia–ischaemia, but after exposure to LPS, increased the importance of the LPS-induced hub (STAT3), while it diminished the relevance of proteins such as the TLR-signalling adapter MyD88, the NFκB subunit p65/RelA,

Table II: Effects of neonatal lipopolysaccharide sensitization on genes related to death receptor signalling, toll-like receptors, NFκB, and p53/apoptosis[86]

Gene name	Fold change at 2h	Fold change at 6h	Protein name and function
Death receptor signalling pathway genes			
Cd40	1.23	1.32	Cluster of differentiation-40, TNF receptor superfamily member 5, mediates chronic inflammation, plays a crucial role in humoral and cellular immunity, and interacts with CD154
Tnf	1.43	1.24	Tumour necrosis factor, TNF superfamily member 2, acts as a cytokine, binds TNF receptors, and plays a role in regulation of cell proliferation, induction of apoptosis, and inflammatory responses
Tnfrsf12a	1.19	1.36	Tumour necrosis factor superfamily member 12a is the receptor for TNF-related weak inducer of apoptosis (TWEAK), a member of the TNF family, and is involved in cell death and adhesion
Ltbr	1.05	1.24	Lymphotoxin beta receptor,TNF receptor superfamily member 3, is involved in positive regulation of the I-κB kinase/NF-κB cascade
Mapk8	1.34	1.08	Mitogen-activated kinase-8 activated by cellular stress and TNFα, involved in inflammation and cell death signalling
Toll-like receptor associated genes			
Cd14	1.42	3.25	CD14 is component of the lipopolysaccharide receptor complex, which mediates LPS-induced neuroinflammation and inflammatory responses
Cxcl10	5.46	7.73	Chemokine (C-X-C motif) ligand 10 is important in TLR-induced inflammation, via TRIF and IRF3, and induces cell migration and proliferation
Fos	2.29	1.77	c-fos, an osteosarcoma oncogene, is an immediate-early gene encoding a subunit of a nuclear transcription factor (AP-1) involved in signal transduction, triggered by TLR activation of Myd88 and TAK-1
Jun	1.35	1.54	Jun oncogene is an immediate-early gene and subunit of a nuclear transcription factor (AP-1) involved in signal transduction triggered by TLR activation of Myd88, JNK, and TAK-1
Map3k7	1.37	1.36	Mitogen-activated protein kinase kinase 7 (TAK1) is activated by Myd88, IRAK1/4, and TRAF6 and triggers IKK, MKK3/6, and MKK4/7 signalling leading to NFκB, JNK, and p38 activation
Myd88	1.13	1.56	Myeloid differentiation primary response gene 88 encodes an adaptor protein which binds TLRs and links them to other downstream signalling molecules
Stat1	1.06	1.56	Signal transducers and activators of transcription factor 1, a component of the IFNγ receptor signalling pathway and JAK–STAT pathway that plays a role in development of cytokine resistance
Tbk1	−1.33	1.35	TANK-binding kinase 1 is activated by TLR activation of TRIF leading to phosphorylation of IRF3 and 7, which in turn trigger production of IFNα and IFNβ
NFκB pathway genes			
Chuk	−1.42	−1.38	Conserved helix–loop–helix ubiquitous kinase, inhibitor of NFκB kinase subunit alpha (IKKα) and part of the NFκB signalling pathway
Nfkbia	3.07	1.72	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha is an inhibitor of NFκB; binds NFκB and retains it in the cytoplasm
Nfkb1	1.20	1.23	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 gene encodes a 105kD polypeptide that can be processed to create a 50kDa protein; the larger 105kDa protein is a Rel protein-specific transcription inhibitor, and the smaller 50kDa protein is a DNA-binding subunit of the NFκB protein complex
Ccl3	1.70	1.26	Chemokine (C-C motif) ligand 3 (MIP-1a) is a NFκB-regulated chemokine that mediates monocyte and neutrophil chemotaxis, and may play a role in the pathogenesis of injury
Il1b	1.97	1.16	Interleukin 1β is a pro-inflammatory cytokine involved in cell injury and oligodendrocyte progenitor cell cycle arrest induced by the MYD88, IRAK 1/4, TRAF6, and TAK1 triggering of NFκB
Il6	1.25	1.06	Interleukin 6 is a cytokine involved in inflammation, injury, and development induced by the Myd88, IRAK 1/4, TRAF6 and TAK1 triggering of NFκB.
p53/apoptosis pathway genes			
Bid	1.21	1.07	BH3-interacting domain death agonist is involved in inducing Bax oligomerization in isolated mitochondrial outer membranes
Birc2	−1.26	−1.25	Baculoviral IAP repeat-containing 2 plays a role in regulation of apoptosis
Birc3	1.64	1.43	Baculoviral IAP repeat-containing 3 (IAP1) inhibits caspases and reduces apoptotic cell death
Casp3	−1.53	−1.70	Apoptotic cysteine–aspartic acid protease that plays a role in regulating neuronal cell death and other apoptotic processes
Cd82	1.22	1.24	p53 target gene (also called Kai1) which is a metastasis-suppressor gene that regulates cell death and cell growth
Cycs	−1.44	−1.38	A component of the electron transport chain in mitochondria, functions in apoptosis by inducing the formation of the apoptosome
Gadd45b	1.64	−1.36	Growth arrest and DNA damage-inducible, beta, is a p53 target gene involved in DNA repair and damage prevention
Igf1	−1.22	−1.23	Insulin-like growth factor-1, which is regulated by p53 and is a major growth factor in the mammalian brain; involved in injury prevention

Table II: Continued

Gene name	Fold change at 2h	Fold change at 6h	Protein name and function
Igfbp3	1.22	2.03	Insulin-like growth factor-binding protein 3 is a p53 target gene that regulates IGF-1 and participates in injury responses
Pik3ca	−1.53	−1.27	Encodes the catalytic, alpha polypeptide of phosphatidylinositol 3-kinase; increased expression of the human homologue of phosphatidylinositol 3-kinase is associated with increased cell proliferation and decreased apoptosis
Pten	−1.59	−1.51	Phosphatase and tensin homologue is a p53 target gene that regulates phosphoinositide 3-kinase- and Akt kinase-mediated anti-apoptotic pathways
Rchy1	−1.22	1.20	Ring finger and CHY zinc finger domain containing 1 is a p53 target gene involved in negative feedback and ubiquitination
Serpine1	1.28	1.28	Serine (or cysteine) peptidase inhibitor, clade E, member 1 (Pai1) is a p53 target gene and promotes neuronal survival

Gene expression was analysed and *t*-tests (corrected using the Benjamini–Hochberg method) were performed. Those genes significantly up- and down-regulated (>1.2-fold, $p<0.05$) in the rat CNS after 2h and 6h of LPS treatment are marked in dark grey or light grey, respectively. H, hour; LPS, lipopolysaccharide; TNF, tumour necrosis factor; TNFR, TNF receptor; TLR, toll-like receptor; IL, interleukin; IGF-1; insulin-like growth factor-1.

TNF-related signalling genes (TRAF5 and TNFRSF1A), and the IL-1 receptor. IDR-1018 treatment also evoked participation of negative regulators of NFκB, suggesting that systemic administration of IDR-1018 can disengage genes that drive LPS-mediated inflammatory responses in the brain. Importantly, IDR-1018 treatment also exerted effects on regulatory molecules of apoptosis/death receptors.[124]

FUTURE DIRECTIONS

Over the last two decades, preclinical studies investigating the safety and efficacy of neuroprotective agents, such as therapeutic hypothermia, have been performed mainly in models of pure hypoxia–ischaemia. Today, in high-income settings, therapeutic hypothermia is an established and safe intervention known to increase disability-free survival amongst affected term infants.[125,126] It is concerning, however, that recent studies suggest reduced effectiveness and possible harm of therapeutic hypothermia in the presence of infection or inflammation in adult clinical studies.[127] In addition, a small preclinical neonatal study found that hypothermia was not effective against hypoxic–ischaemic injury under conditions of LPS sensitization.[128] However, this study assessed the effects of hypothermia in infants with very severe injury (30% infarct). As such, more needs to be known about the utility of hypothermia in models of injury more closely representing those seen in the majority of infants treated with hypothermia, in a complex and strongly translational model.

In addition, to understand the role of inflammation-sensitized injury to the brain in term infants, and for therapy development, there are several key questions that require additional clinical and preclinical research, as follows: (1) What is the effect of infection and inflammation on the pattern of brain injury seen in neonatal encephalopathy, what is its role in impairment outcomes in both high- and low-income settings and is there an association between the severity of inflammation or its presence and outcomes? (2) Can we improve our understanding of the relationship between fetal and maternal genetics to identify at-risk pregnancies? (3) Can we identify early biomarkers of inflammation and injury severity from clinically accessible fluids (e.g. urine, CSF, or blood)? Using biomarkers, can we acquire a better understanding of the temporal progression of the innate and adaptive immune response to brain injury, and how this is altered with inflammation sensitization and hypothermia? Will monitoring of additional maternal and fetal observations of inflammatory status, including any infectious serotype, support therapy design and implementation?

CONCLUSION

There is increasing evidence of a strong association between inflammatory sensitization and neonatal encephalopathy. This association is observed in different resource settings and, as part of the burgeoning interest in inflammation and infection, work is being done to further characterize these processes. The implementation of hypothermia has been an immense achievement, although it reduces the burden of disability by only 11%. An appreciation of underlying sensitizing events might be an important step towards the design of new and/or adjunct therapies to further reduce the burden of disability in infants suffering neonatal encephalopathy.

ACKNOWLEDGEMENTS

The research of the authors is supported by the Inserm (PG), Paris Diderot University (PG), the PremUP Foundation (PG), the Seventh Framework Program of the European Union (grant agreement No HEALTH-F2-2009-241778/Neurobid to PG and CM), the Indo-French Centre for the Promotion of Advanced Research (CEFIPRA project No. 4903-H to PG), the Leducq Foundation (HH, PG and CM), the de Spoelberch Foundation (PG), the Grace de Monaco Foundation (PG), Medical Research Council Sweden (2012-2642 to HH and 2012-2992 to CM), ALF-LUA (ALFGBG2863 to HH and ALFGBG-142881 to CM), Wellcome Trust Program Grant (WT094823MA to HH and PG) and Wellcome Trust Clinical PhD Training Fellowship (094016/Z/10/Z to CT), Medical Research Council UK (NR), Wellbeing of Women (NR), Action Medical Research (NR), Royal Society

Wolfson Research Foundation (NR), UK Department of Health's National Institute for Health Research Biomedical Research Centres funding scheme, the Assistance Publique-Hôpitaux de Paris ('contrat hospitalier de recherche translationnelle' to PG), the Åhlén Foundation (CM), Frimurare Barnhusfonden (CM), Foundation Olle Engkvist Byggmästare (CM), Swedish Brain Foundation (FO2013-095 to CM) and Wilhelm and Martina Lundgren Foundation (CM), and the Research Foundation of Cerebral Palsy Alliance (PG and BF). The authors have stated that they had no interests that might be perceived as posing a conflict or bias.

REFERENCES

1. You D, Bastian P, Wu J, Wardlaw T. Levels & Trends in Child Mortality. New York: WHO, World Bank, UN-DESA Population Division, UNICEF, New York. 2013.
2. Lee ACC, Kozuki K, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at the regional and global levels for 2010 with trends from 1990. *Pediatr Res* 2013; **74**: 50–72.
3. Nelson KB, Leviton A. How much of neonatal encephalopathy is due to birth asphyxia? *Am J Dis Child* 1991; **145**: 1323–31.
4. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic–ischaemic encephalopathy. *Early Hum Dev* 2010; **86**: 329–38.
5. Gunn AJ, Gluckman PD, Gunn TR. Selective head cooling in newborn infants after perinatal asphyxia: a safety study. *Pediatrics* 1998; **102**: 885–92.
6. Gunn AJ, Parer JT, Mallard EC, Williams CE, Gluckman PD. Cerebral histological and electrocorticographic changes after asphyxia in fetal sheep. *Pediatr Res* 1992; **31**: 486–91.
7. Badawi N, Kurinczuk JJ, Keogh JM, et al. Antepartum risk factors for newborn encephalopathy: the Western Australian case–control study. *Br Med J* 1998; **317**: 1549–53.
8. Badawi N, Kurinczuk JJ, Keogh JM, et al. Intrapartum risk factors for newborn encephalopathy: the Western Australian case–control study. *Br Med J* 1998; **317**: 1554–8.
9. Ellis M, Manandhar N, Manandhar DS, Costello AM. Risk factors for neonatal encephalopathy in Kathmandu, Nepal, a developing country: unmatched case–control study. *Br Med J* 2000; **320**: 1229–36.
10. Martinez-Biarge M, Diez-Sebastian J, Wustoff CW, Mercuri E, Cowan FM. Antepartum and intrapartum factors preceding neonatal hypoxic–ischemic encephalopathy. *Pediatrics* 2013; **132**: e952–9.
11. Ellis M, Costello AM. Antepartum risk factors for newborn encephalopathy. Intrapartum risk factors are important in developing world. *Br Med J* 1999; **318**: 1414 (author reply 5).
12. Peebles DM, Wyatt JS. Synergy between antenatal exposure to infection and intrapartum events in causation of perinatal brain injury at term. *BJOG* 2002; **109**: 737–9.
13. Hagberg H, Gressens P, Mallard C. Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. *Ann Neurol* 2012; **71**: 444–57.
14. Kendall G, Peebles D. Acute fetal hypoxia: the modulating effect of infection. *Early Hum Dev* 2005; **81**: 27–34.
15. Foster-Barber A, Ferriero DM. Neonatal encephalopathy in the term infant: neuroimaging and inflammatory cytokines. *Ment Retard Dev Disabil Res Rev* 2002; **8**: 20–4.
16. Wang X, Hagberg H, Nie C, et al. Dual role of intrauterine immune challenge on neonatal and adult brain vulnerability to hypoxia–ischemia. *J Neuropathol Exp Neurol* 2007; **66**: 552–61.
17. Rousset CI, Kassem J, Olivier P, et al. Antenatal bacterial endotoxin sensitizes the immature rat brain to postnatal excitotoxic injury. *J Neuropathol Exp Neurol* 2008; **67**: 994–1000.
18. Rangon CM, Fortes S, Lelievre V, et al. Chronic mild stress during gestation worsens neonatal brain lesions in mice. *J Neurosci* 2007; **27**: 7532–40.
19. Olivier P, Baud O, Bouslama M, et al. Moderate growth restriction: deleterious and protective effects on white matter damage. *Neurobiol Dis* 2007; **26**: 253–63.
20. Dommergues MA, Patkai J, Renauld JC, Evrard P, Gressens P. Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. *Ann Neurol* 2000; **47**: 54–63.
21. Eklind S, Mallard C, Leverin AL, et al. Bacterial endotoxin sensitizes the immature brain to hypoxic–ischaemic injury. *Eur J Neurosci* 2001; **13**: 1101–6.
22. Fontaine RH, Olivier P, Massonneau V, et al. Vulnerability of white matter towards antenatal hypoxia is linked to a species-dependent regulation of glutamate receptor subunits. *Proc Nalt Acad Sci USA* 2008; **105**: 16779–84.
23. Romero R, Velez Edwards DR, Kusanovic JP, et al. Identification of fetal and maternal single nucleotide polymorphisms in candidate genes that predispose to spontaneous preterm labor with intact membranes. *Am J Obstet Gynecol* 2010; **202**: 431.e1–34.
24. Nelson KB, Dambrosia JM, Grether JK, Phillips TM. Neonatal cytokines and coagulation factors in children with cerebral palsy. *Ann Neurol* 1998; **44**: 665–75.
25. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. *Pediatr Res* 1997; **42**: 1–8.
26. Lin CY, Chang YC, Wang ST, et al. Altered inflammatory responses in preterm children with cerebral palsy. *Ann Neurol* 2010; **68**: 204–12.
27. Kasdorf E, Perlman JM. Hyperthermia, inflammation, and perinatal brain injury. *Pediatr Neurol* 2013; **49**: 8–14.
28. Menon R, Taylor RN, Fortunato SJ. Chorioamnionitis – a complex pathophysiologic syndrome. *Placenta* 2010; **31**: 113–20.
29. Blume H, Li C, Loch CM, Koepsell TD. Intrapartum fever and choriamnionitis as risks for encephalopathy in term newborns: a case–control study. *Dev Med Child Neurol* 2008; **50**: 19–24.
30. Redline RW. Inflammatory responses in the placenta and umbilical cord. *Semin Fetal Neonatal Med* 2006; **11**: 296–301.
31. Impey L, Greenwood C, MacQuillan K, Reynolds M, Sheil O. Fever in labour and neonatal encephalopathy: a prospective cohort study. *BJOG* 2001; **108**: 594–7.
32. Impey LW, Greenwood CE, Black RS, et al. The relationship between intrapartum maternal fever and neonatal acidosis as risk factors for neonatal encephalopathy. *Am J Obstet Gynecol* 2008; **198**: 49.e1–6.
33. Nelson KB, Grether JK. Potentially asphyxiating conditions and spastic cerebral palsy in infants of normal birth weight. *Am J Obstet Gynecol* 1998; **179**: 507–13.
34. Nelson KB, Ellenberg JH. Epidemiology of cerebral palsy. *Adv Neurol* 1978; **19**: 421–35.
35. Peebles D. Intrauterine Infection and Perinatal Brain Injury. Scientific Advisory Committee Opinion Paper. London: Royal College of Obstetrics & Gynecology, 2007.
36. Chalak L, Sánchez P, Adams-Huet B, et al. Biomarkers for severity of neonatal hypoxic–ischemic encephalopathy and outcomes in newborns receiving hypothermia therapy. *J Pediatr* 2013; **162**: 35–41.
37. Liu F, McCullough LD. Inflammatory responses in hypoxic ischemic encephalopathy. *Acta Pharmacol Sin* 2013; **34**: 1121–30.
38. Shalak LF, Laptook AR, Jafri HS, Ramilo O, Perlman JM. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. *Pediatrics* 2002; **110**: 673–80.
39. Iwamoto A, Seward N, Prost A, et al. Maternal infection and risk of intrapartum death: a population based observational study in South Asia. *BMC Pregnancy Childbirth* 2013; **13**: 245.
40. Nelson KB, Bingham P, Edwards EM, et al. Antecedants of Neonatal Encephalopathy in the Vermont Oxford Network Emecephalopathy Register. *Pediatrics* 2012; **130**: 878.
41. Jenster M, Bonifacio S, Ruel T, et al. Maternal or neonatal infection: association with neonatal encephalopathy outcomes. *Pediatr Res* 2014; **76**: 93–9.
42. Jacobs S, Berg M, Hunt R, Tarnow-Mordi W, Inder T, Davis P. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2013; **1**: CD003311.
43. Houben ML, Nikkels PG, van Bleek GM, et al. The association between intrauterine inflammation and

spontaneous vaginal delivery at term: a cross-sectional study. *PLoS One* 2009; **10**: e6572.

44. Bartha AI, Foster-Barber A, Miller SP, et al. Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. *Pediatr Res* 2004; **56**: 960–6.

45. Wu YW, Colford JM. Chorioamnionitis as a risk factor for cerebral palsy; a meta-analysis. *J Am Med Assoc* 2000; **284**: 1417–24.

46. Redline RW, O'Riordan MA. Placental lesions associated with cerebral palsy and neurologic impairment following term birth. *Arch Pathol Lab Med* 2000; **124**: 1785–91.

47. Harteman JC, Nikkels PG, Benders MJ, et al. Placental pathology in full-term infants with hypoxic–ischemic neonatal encephalopathy and association with magnetic resonance imaging pattern of brain injury. *J Pediatr* 2013; **163**: 968–95.e2.

48. Tann CJ, Nakakeeto M, Kutinczuk JJ, et al. The Neonatal Society Summer Meeting. Edinburgh: John McIntyre Conference Centre, Pollock Halls, 2013. http://www.neonatalsociety.ac.uk/meetings/2013_summer_abstracts.pdf.

49. Hayes B, Cooley S, Donnelly J, et al. The placenta in infants >36 weeks gestation with neonatal encephalopathy: a case control study. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**: F233–40.

50. Wintermark P, Boyd T, Gregas MC, Labrecque M, Hansen A. Placental pathology in apshyxiated newborns meeting the criteria for therapeutic hypothermia. *Am J Obstet Gynecol* 2010; **203**: 597.e1–9.

51. Robertson NJ, Nakakeeto M, Hagmann C, et al. Therapeutic hypothermia for birth asphyxia in low-resource settings: a pilot randomised controlled trial. *Lancet* 2008; **372**: 801–3.

52. Redline RW. Placental Pathology: a systematic approach with clinical correlations. *Placenta* 2008; **22**: S86–91.

53. Boog G. Chronic villitis of unknown etiology. *Eur J Obstet Gynecol Reprod Biol* 2008; **136**: 9–15.

54. Rice JE 3rd, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic–ischemic brain damage in the rat. *Ann Neurol* 1981; **9**: 131–41.

55. Bona E, Andersson AL, Blomgren K, et al. Chemokine and inflammatory cell response to hypoxia–ischemia in immature rats. *Pediatr Res* 1999; **45**: 500–9.

56. Marret S, Mukendi R, Gadisseux JF, Gressens P, Evrard P. Effect of ibotenate on brain development: an excitotoxic mouse model of microgyria and posthypoxic-like lesions. *J Neuropathol Exp Neurol* 1995; **54**: 358–70.

57. Dicou E, Rangon CM, Guimiot F, Spedding M, Gressens P. Positive allosteric modulators of AMPA receptors are neuroprotective against lesions induced by an NMDA agonist in neonatal mouse brain. *Brain Res* 2003; **970**: 221–5.

58. Stridh L, Mottahedin A, Johansson ME, et al. Toll-like receptor-3 activation increases the vulnerability of the neonatal brain to hypoxia–ischemia. *J Neurosci* 2013; **33**: 12041–51.

59. Wang X, Stridh L, Li W, et al. Lipopolysaccharide sensitizes neonatal hypoxic–ischemic brain injury in a MyD88-dependent manner. *J Immunol* 2009; **183**: 7471–7.

60. Fleiss B, Nilsson MK, Blomgren K, Mallard C. Neuroprotection by the histone deacetylase inhibitor trichostatin A in a model of lipopolysaccharide-sensitised neonatal hypoxic–ischaemic brain injury. *J Neuroinflammation* 2012; **9**: 70.

61. Patkai J, Mesples B, Dommergues MA, et al. Deleterious effects of IL-9-activated mast cells and neuroprotection by antihistamine drugs in the developing mouse brain. *Pediatr Res* 2001; **50**: 222–30.

62. Eklind S, Mallard C, Arvidsson P, Hagberg H. Lipopolysaccharide induces both a primary and a secondary phase of sensitization in the developing rat brain. *Pediatr Res* 2005; **58**: 112–6.

63. Lin HY, Huang CC, Chang KF. Lipopolysaccharide preconditioning reduces neuroinflammation against hypoxic ischemia and provides long-term outcome of neuroprotection in neonatal rat. *Pediatr Res* 2009; **66**: 254–9.

64. Favrais G, Schwendimann L, Gressens P, Lelievre V. Cyclooxygenase-2 mediates the sensitizing effects of systemic IL-1-beta on excitotoxic brain lesions in newborn mice. *Neurobiol Dis* 2007; **25**: 496–505.

65. Aden U, Favrais G, Plaisant F, et al. Systemic inflammation sensitizes the neonatal brain to excitotoxicity through a pro-/anti-inflammatory imbalance: key role of TNFalpha pathway and protection by etanercept. *Brain Behav Immun* 2010; **24**: 747–58.

66. Yang L, Sameshima H, Yamaguchi M, Ikenoue T. Expression of inducible nitric oxide synthase and cyclooxygenase-2 mRNA in brain damage induced by lipopolysaccharide and intermittent hypoxia–ischemia in neonatal rats. *J Obstet Gynaecol Res* 2005; **31**: 185–91.

67. Girard S, Sebire H, Brochu ME, et al. Postnatal administration of IL-1Ra exerts neuroprotective effects following perinatal inflammation and/or hypoxic–ischemic injuries. *Brain Behav Immun* 2012; **26**: 1331–9.

68. Kendall GS, Hristova M, Horn S, et al. TNF gene cluster deletion abolishes lipopolysaccharide-mediated sensitization of the neonatal brain to hypoxic ischemic insult. *Lab Invest* 2011; **91**: 328–41.

69. Van SJ, Schang AL, Chhor V, et al. 11th European Meeting on Glial Cell Function in Health and Disease, Berlin, Germany, 2013.

70. Winerdal M, Winerdal ME, Kinn J, et al. Long lasting local and systemic inflammation after cerebral hypoxic ischemia in newborn mice. *PLoS One* 2012; **7**: e36422.

71. Shanks N, Windle RJ, Perks PA, et al. Early-life exposure to endotoxin alters hypothalamic-pituitary-adrenal function and predisposition to inflammation. *Proc Natl Acad Sci USA* 2000; **97**: 5645–50.

72. Ikeda T, Yang L, Ikenoue T, Mallard C, Hagberg H. Endotoxin-induced hypoxic–ischemic tolerance is mediated by up-regulation of corticosterone in neonatal rat. *Pediatr Res* 2006; **59**: 56–60.

73. Ikeda T, Mishima K, Aoo N, et al. Dexamethasone prevents long-lasting learning impairment following a combination of lipopolysaccharide and hypoxia–ischemia in neonatal rats. *Am J Obstet Gynecol* 2005; **192**: 719–26.

74. Degos V, Peineau S, Nijboer C, et al. G protein-coupled receptor kinase 2 and group I metabotropic glutamate receptors mediate inflammation-induced sensitization to excitotoxic neurodegeneration. *Ann Neurol* 2013; **73**: 667–78.

75. Reiter E, Lefkowitz RJ. GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab* 2006; **17**: 159–65.

76. Alves-Filho JC, Sonego F, Souto FO, et al. Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nat Med* 2010; **16**: 708–12.

77. Nijboer CH, Heijnen CJ, Willemen HL, et al. Cell-specific roles of GRK2 in onset and severity of hypoxic–ischemic brain damage in neonatal mice. *Brain Behav Immun* 2010; **24**: 420–6.

78. Lehnardt S, Lachance C, Patrizi S, et al. The toll-like receptor TLR4 is necessary for lipopolysaccharide-induced oligodendrocyte injury in the CNS. *J Neurosci* 2002; **22**: 2478–86.

79. Moll UM, Wolff S, Speidel D, Deppert W. Transcription-independent pro-apoptotic functions of p53. *Curr Opin Cell Biol* 2005; **17**: 631–6.

80. Green DR, Kroemer G. Cytoplasmic functions of the tumour suppressor p53. *Nature* 2009; **458**: 1127–30.

81. Nijboer CHA, Heijnen CJ, Groenendaal F, et al. Strong neuroprotection by inhibition of NF-kappaB after neonatal hypoxia–ischemia involves apoptotic mechanisms but is independent of cytokines. *Stroke* 2008; **39**: 2129–37.

82. Dean JM, Wang X, Kaindl AM, et al. Microglial MyD88 signaling regulates acute neuronal toxicity of LPS-stimulated microglia in vitro. *Brain Behav Immun* 2010; **24**: 776–83.

83. Yang D, Sun YY, Lin X, et al. Intranasal delivery of cell-penetrating anti-NF-kappaB peptides (Tat-NBD) alleviates infection-sensitized hypoxic–ischemic brain injury. *Exp Neurol* 2013; **247**: 447–55.

84. Eklind S, Hagberg H, Wang X, et al. Effect of lipopolysaccharide on global gene expression in the immature rat brain. *Pediatr Res* 2006; **60**: 161–8.

85. Hagberg H, Mallard C. Effect of inflammation on central nervous system development and vulnerability. *Curr Opin Neurol* 2005; **18**: 117–23.

86. Taylor DL, Jones F, Kubota ES, Pocock JM. Stimulation of microglial metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced neurotoxicity in concert with microglial-derived Fas ligand. *J Neurosci* 2005; **25**: 2952–64.

87. Hoffmann O, Zipp F, Weber JR. Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in central nervous system inflammation. *J Mol Med* 2009; **87**: 753–63.

88. Yepes M, Brown SAN, Moore EG, et al. A soluble Fn14-Fc decoy receptor reduces infarct volume in a murine model of cerebral ischemia. *Am J Pathol* 2005; **166**: 511–20.

89. Aktas O, Schulze-Topphoff U, Zipp F. The role of TRAIL/TRAIL receptors in central nervous system pathology. *Front Biosci* 2007; **12**: 2912–21.

90. Thornton C, Rousset CI, Kichev A, et al. Molecular mechanisms of neonatal brain injury. *Neurol Res Int* 2012; **2012**: 506320.

91. Markus T, Cronberg T, Cilio C, et al. Tumor necrosis factor receptor-1 is essential for LPS-induced sensitization and tolerance to oxygen-glucose deprivation in murine neonatal organotypic hippocampal slices. *J Cerebr Blood F Met* 2009; **29**: 73–86.

92. Yu C, Takeda M, Soliven B. Regulation of cell cycle proteins by TNF-alpha and TGF-beta in cells of oligodendroglial lineage. *J Neuroimmunol* 2000; **108**: 2–10.

93. Andrews T, Zhang P, Bhat NR. TNFalpha potentiates IFNgamma-induced cell death in oligodendrocyte progenitors. *J Neurosci Res* 1998; **54**: 574–83.

94. Selmaj KW, Farooq M, Norton WT, Raine CS, Brosnan CF. Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. *J Immunol* 1990; **144**: 129–35.

95. Dopp JM, Mackenzie-Graham A, Otero GC, Merrill JE. Differential expression, cytokine modulation, and specific functions of type-1 and type-2 tumor necrosis factor receptors in rat glia. *J Neuroimmunol* 1997; **75**: 104–12.

96. Genc S, Kizildag S, Genc K, Ates H, Atabey N. Interferon gamma and lipopolysaccharide upregulate TNF-related apoptosis-inducing ligand expression in murine microglia. *Immunol Lett* 2003; **85**: 271–4.

97. Cui M, Wang L, Liang X, et al. Blocking TRAIL-DR5 signaling with soluble DR5 reduces delayed neuronal damage after transient global cerebral ischemia. *Neurobiol Dis* 2010; **39**: 138–47.

98. Kitchev A, Thornton C, Rousset C, et al. TRAIL signaling and cell death in the immature CNS after hypoxia–ischemia and inflammation. *J Biol Chem* 2014; **289**: 9430–9.

99. Song HY, Régnier CH, Kirschning CJ, Goeddel DV, Rothe M. Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun N-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc Nat Acad Sci USA* 1997; **94**: 9792–6.

100. Winkles JA. The TWEAK-Fn14 cytokine–receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov* 2008; **7**: 411–25.

101. Lehnardt S, Massillon L, Follett P, et al. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc Natl Acad Sci USA* 2003; **100**: 8514–9.

102. Mesples B, Fontaine RH, Lelievre V, Launay JM, Gressens P. Neuronal TGF-beta1 mediates IL-9/mast cell interaction and exacerbates excitotoxicity in newborn mice. *Neurobiol Dis* 2005; **18**: 193–205.

103. Robertson NJ, Tan S, Groenendaal F, et al. Which neuroprotective agents are ready for bench to bedside translation in the newborn infant? *J Pediatr* 2012; **160**: 544–52.e4.

104. Gonzalez FF, Ferriero DM. Neuroprotection in the newborn infant. *Clin Perinatol* 2009; **36**: 859–80, vii.

105. Johnston MV, Fatemi A, Wilson MA, Northington F. Treatment advances in neonatal neuroprotection and neurointensive care. *Lancet Neurol* 2011; **10**: 372–82.

106. Sugimoto J, Romani AM, Valentin-Torres AM, et al. Magnesium decreases inflammatory cytokine production: a novel innate immunomodulatory mechanism. *J Immunol* 2012; **188**: 6338–46.

107. Burd I, Breen K, Friedman A, Chai J, Elovitz MA. Magnesium sulfate reduces inflammation-associated brain injury in fetal mice. *Am J Obstet Gynecol* 2010; **202**: 292.e1–9.

108. Doyle LW, Crowther CA, Middleton P, Marret S. Antenatal magnesium sulfate and neurologic outcome in preterm infants: a systematic review. *Obstet Gynecol* 2009; **113**: 1327–33.

109. Kimura A, Sakurada S, Ohkuni H, Todome Y, Kurata K. Moderate hypothermia delays proinflammatory cytokine production of human peripheral blood mononuclear cells. *Crit Care Med* 2002; **30**: 1499–502.

110. Polderman KH. Hypothermia, immune suppression and SDD: can we have our cake and eat it? *Crit Care* 2011; **15**: 144.

111. Czeh M, Gressens P, Kaindl AM. The yin and yang of microglia. *Dev Neurosci* 2011; **33**: 199–209.

112. Doring A, Yong VW. The good, the bad and the ugly. Macrophages/microglia with a focus on myelin repair. *Front Biosci (Schol Ed)* 2011; **3**: 846–56.

113. Jenkins D, Lee T, Chiuzan C, et al. Altered circulating leukocytes and their chemokines in a clinical trial of therapeutic hypothermia for neonatal hypoxic ischemic encephalopathy. *Pediatr Crit Care Med* 2013; **14**: 786–95.

114. Robertson N, Faulkner S, Fleiss B, et al. Melatonin Augments Hypothermic Neuroprotection in a Perinatal Asphyxia Piglet Model. *Brain* 2013; **136**: 90–105.

115. Pei Z, Pang S, Cheung R. Administration of melatonin after onset of ischemia reduces the volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model. *Stroke* 2003; **34**: 770–5.

116. Gerber J, Lotz M, Ebert S, et al. Melatonin is neuroprotective in experimental Streptococcus pneumoniae meningitis. *J Infect Dis* 2005; **19**: 783–90.

117. Halili MA, Andrews MR, Labzin LI, et al. Differential effects of selective HDAC inhibitors on macrophage inflammatory responses to the Toll-like receptor 4 agonist LPS. *J Leukoc Biol* 2010; **87**: 1103–14.

118. Kim HJ, Rowe M, Ren M, et al. Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: multiple mechanisms of action. *J Pharmacol Exp Ther* 2007; **321**: 892–901.

119. Suh HS, Choi S, Khattar P, Choi N, Lee SC. Histone deacetylase inhibitors suppress the expression of inflammatory and innate immune response genes in human microglia and astrocytes. *J Neuroimmune Pharmacol* 2010; **5**: 521–32.

120. Zhang B, West EJ, Van KC, et al. HDAC inhibitor increases histone H3 acetylation and reduces microglia inflammatory response following traumatic brain injury in rats. *Brain Res* 2008; **1226**: 181–91.

121. Fischer A, Sananbenesi F, Mungenast A, Tsai LH. Targeting the correct HDAC(s) to treat cognitive disorders. *Trends Pharmacol Sci* 2010; **31**: 605–17.

122. Butler KV, Kalin J, Brochier C, et al. Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A. *J Am Chem Soc* 2010; **132**: 10842–6.

123. Yang D, Sun YY, Nemkul N, et al. Plasminogen activator inhibitor-1 mitigates brain injury in a rat model of infection-sensitized neonatal hypoxia–ischemia. *Cereb Cortex* 2013; **23**: 1218–29.

124. Bolouri H, Savman K, Wang W, et al. Innate defence regulator peptide 1018 protects against perinatal brain injury. *Ann Neurol* 2013; **75**: 395–410.

125. Azzopardi DV, Strohm B, Edwards AD, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009; **361**: 1349–58.

126. Shankaran S, Laptook AR, Ehrenkranz RA, et al. Whole-body hypothermia for infants with hypoxic ischaemic encephalopathy. *N Engl J Med* 2005; **353**: 1574–84.

127. Mourvillier B, Tubach F, van der Beek D, et al. Induced hypothermia in severe bacterial meningitis. *J Am Med Assoc* 2013; **310**: 2174–83.

128. Osredkar D, Thoresen M, Maes E, Flatebo T, Elstad M, Sabir H. Hypothermia is not neuroprotective after infection-sensitized neonatal hypoxic–ischemic brain injury. *Resuscitation* 2014; **85**: 567–72.